

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>A61K 31/585</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 96/24358</b> <b>(43) International Publication Date:</b> 15 August 1996 (15.08.96)
<b>(21) International Application Number:</b> PCT/US96/01769 <b>(22) International Filing Date:</b> 9 February 1996 (09.02.96) <b>(30) Priority Data:</b> 08/386,793 10 February 1995 (10.02.95) US <b>(60) Parent Application or Grant</b> <b>(63) Related by Continuation</b> US 08/386,793 (CIP) Filed on 10 February 1995 (10.02.95) <b>(71) Applicant (for all designated States except US):</b> G.D. SEARLE & CO. [US/US]; Corporate Patent Dept., P.O. Box 5110, Chicago, IL 60680-5110 (US). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> MACLAUGHLAN, Todd, E. [CA/US]; 34128 Sulkey Drive, Grayslake, IL 60030 (US). PEREZ, Alfonso, T. [US/US]; 1286 Cascade Court, Lake Forest, IL 60045 (US). <b>(74) Agents:</b> KEANE, J., Timothy et al.; G.D. Searle & Co., Corporate Patent Dept., P.O. Box 5110, Chicago, IL 60680-5110 (US).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AZ, BY, KG, KZ, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> USE OF LOW DOSE AMOUNT OF SPIRONOLACTONE FOR TREATMENT OF CARDIOVASCULAR DISEASE  <b>(57) Abstract</b>  Use of low dosages of an aldosterone receptor antagonist, spironolactone, is described for treatment of circulatory disorders. This therapy would be particularly useful to treat or retard the development of congestive heart failure, while avoiding or reducing aldosterone-antagonist-induced side effects, such as hyperkalemia.		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

USE OF LOW DOSE AMOUNT OF SPIRONOLACTONE FOR  
TREATMENT OF CARDIOVASCULAR DISEASE

5

Field of the Invention

Spironolactone, an aldosterone receptor antagonist, is described for use in treatment of circulatory disorders, including cardiovascular diseases such as heart failure, hypertension and congestive heart failure. Of particular interest is a therapy using spironolactone at a low dosage at which side-effects are reduced or avoided.

15

Background of the Invention

Myocardial (or cardiac) failure, that is, heart failure ("HF"), whether a consequence of previous myocardial infarction(s), heart disease associated with hypertension, or primary cardiomyopathy, is a major health problem of worldwide proportions. The incidence of symptomatic heart failure has risen steadily over the past several decades.

25

In clinical terms, decompensated cardiac failure consists of a constellation of signs and symptoms that arise from congested organs and hypoperfused tissues to form congestive heart failure (CHF) syndrome. Congestion is caused largely by increased venous pressure and by inadequate sodium (Na<sup>+</sup>) excretion, relative to dietary Na<sup>+</sup> intake, and is importantly related to circulating levels of aldosterone (ALDO). An abnormal retention of Na<sup>+</sup> occurs via tubular epithelial cells throughout the nephron, including the later portion of the distal tubule and cortical collecting ducts, where ALDO receptor sites are present.

ALDO is the body's most potent mineralocorticoid hormone. As connoted by the term mineralocorticoid, this steroid hormone has mineral-regulating activity. It promotes Na<sup>+</sup> reabsorption not only in the kidney, but also  
5 from the lower gastrointestinal tract and salivary and sweat glands, each of which represents classic ALDO-responsive tissues. ALDO regulates Na<sup>+</sup> and water resorption at the expense of potassium (K<sup>+</sup>) and magnesium (Mg<sup>2+</sup>) excretion.

10

ALDO can also provoke responses in non-epithelial cells. Elicited by a chronic elevation in plasma ALDO level that is inappropriate relative to dietary Na<sup>+</sup> intake, these responses can have adverse consequences on  
15 the structure of the cardiovascular system. Hence, ALDO can contribute to the progressive nature of myocardial failure for multiple reasons.

Multiple factors regulate ALDO synthesis and  
20 metabolism, many of which are operative in the patient with myocardial failure. These include renin as well as non-renin-dependent factors (such as K<sup>+</sup>, ACTH) that promote ALDO synthesis. Hepatic blood flow, by regulating the clearance of circulating ALDO, helps determine ALDO plasma  
25 concentration, an important factor in heart failure characterized by reduction in cardiac output and hepatic blood flow.

The renin-angiotensin-aldosterone system ("RAAS") is  
30 one of the hormonal mechanisms involved in regulating pressure/volume homeostasis and also in the development of hypertension, a precursor condition implicated in the progression of more serious cardiovascular diseases such as congestive heart failure. Activation of the renin-  
35 angiotensin-aldosterone system begins with secretion of the enzyme renin from the juxtaglomerular cells in the kidney. The enzyme renin acts on a naturally-occurring substrate,

angiotensinogen, to release a decapeptide, Angiotensin I. This decapeptide is cleaved by angiotensin converting enzyme ("ACE") to provide an octapeptide, Angiotensin II, the primary active species of this system. This  
5 octapeptide, angiotensin II, is a potent vasoconstrictor and also produces other physiological effects such as stimulating aldosterone secretion, promoting sodium and fluid retention, inhibiting renin secretion, increasing sympathetic nervous system activity, stimulating  
10 vasopressin secretion, causing positive cardiac inotropic effect and modulating other hormonal systems.

Emphasis has been placed on minimizing hyperaldosteronism as a basis for optimizing patient  
15 treatment. This includes the importance of ALDO-receptor antagonism both in patients treated with conventional diuretic programs and in patients treated with angiotensin-converting enzyme (ACE) inhibitors, who are often constrained to small doses of ACE inhibitor because of  
20 orthostatic hypotension. Such patients may demonstrate a recurrence of heart failure symptoms likely related to elevations in plasma ALDO levels.

Many aldosterone receptor blocking drugs and their  
25 effects in humans are known. For example, spironolactone is a drug which acts at the mineralocorticoid receptor level by competitively inhibiting aldosterone binding. This steroidal compound has been used for blocking aldosterone-dependent sodium transport in the distal tubule  
30 of the kidney in order to reduce edema and to treat essential hypertension and primary hyperaldosteronism [F. Mantero et al, Clin. Sci. Mol. Med., 45 (Suppl 1), 219s-224s (1973)]. Spironolactone is also used commonly in the treatment of other hyperaldosterone-related diseases such  
35 as liver cirrhosis and congestive heart failure [F.J. Saunders et al, Aldactone: Spironolactone: A Comprehensive Review, Searle, New York (1978)]. Progressively-increasing

doses of spironolactone from 1 mg to 400 mg per day [i.e., 1 mg/day, 5 mg/day, 20 mg/day] was administered to a spironolactone-intolerant patient to treat cirrhosis-related ascites [P.A. Greenberger et al, N. Eng. Reg. Allergy Proc., 7(4), 343-345 (Jul-Aug, 1986)]. It has been recognized that development of myocardial fibrosis is sensitive to circulating levels of both Angiotensin II and aldosterone, and that the aldosterone antagonist spironolactone prevents myocardial fibrosis in animal models, thereby linking aldosterone to excessive collagen deposition [D. Klug et al, Am. J. Cardiol., 71(3), 46A-54A (1993)]. Spironolactone has been shown to prevent fibrosis in animal models irrespective of the development of left ventricular hypertrophy and the presence of hypertension [C.G. Brilla et al, J. Mol. Cell. Cardiol., 25(5), 563-575 (1993)]. Spironolactone at a dosage ranging from 25 mg to 100 mg daily is used to treat diuretic-induced hypokalemia, when orally-administered potassium supplements or other potassium-sparing regimens are considered inappropriate [Physicians' Desk Reference, 46th Edn., p. 2153, Medical Economics Company Inc., Montvale, N.J. (1992)].

Previous studies have shown that inhibiting ACE inhibits the renin-angiotensin system by substantially complete blockade of the formation of Angiotensin II. Many ACE inhibitors have been used clinically to control hypertension. While ACE inhibitors may effectively control hypertension, side effects are common including chronic cough, skin rash, loss of taste sense, proteinuria and neutropenia.

Moreover, although ACE inhibitors effectively block the formation of Angiotensin II, aldosterone levels are not well controlled in certain patients having cardiovascular diseases. For example, despite continued ACE inhibition in hypertensive patients receiving captopril, there has been observed a gradual return of plasma aldosterone to baseline

levels [J. Staessen et al, J. Endocrinol., 91, 457-465 (1981)]. A similar effect has been observed for patients with myocardial infarction receiving zofenopril [C. Borghi et al, J. Clin. Pharmacol., 33, 40-45 (1993)]. This  
5 phenomenon has been termed "aldosterone escape".

In a side-by-side treatment of two cohorts of rats, one cohort treated with spironolactone subcutaneously and the other cohort treated with captopril,  
10 spironolactone was found to prevent fibrosis in the hypertensive-rat cohort [C.G. Brilla et al, J. Mol. Cell. Cardiol., 25, 563-575 (1993)].

### Summary of Drawing Figures

Fig. 1 shows urinary aldosterone levels at different  
5 rates of spironolactone administration (12.5 mg,  
25 mg, 50mg, 75mg), as compared to placebo, co-administered  
with stable doses of ACE inhibitor and loop diuretic.

Fig. 2 shows plasma renin activity at different rates  
10 of spironolactone administration (12.5 mg, 25 mg, 50mg, 75mg),  
as compared to placebo, co-administered with stable doses  
of ACE inhibitor and loop diuretic.

Fig. 3 shows N-Terminal ANF levels at different rates  
15 of spironolactone administration (12.5 mg,  
25 mg, 50mg, 75mg), as compared to placebo, co-administered  
with stable doses of ACE inhibitor and loop diuretic.

Fig. 4 shows changes in supine blood pressure at  
20 different rates of spironolactone administration (12.5  
mg, 25 mg, 50mg, 75mg), as compared to placebo,  
co-administered with stable doses of ACE inhibitor and loop  
diuretic.

25 Fig. 5 shows changes in supine heart rate at different  
rates of spironolactone administration (12.5 mg, 25  
mg, 50mg, 75mg), as compared to placebo, co-administered with  
stable doses of ACE inhibitor and loop diuretic.



Description of the Invention

Treatment or prevention of circulatory disorders, including cardiovascular disorders such as heart failure, hypertension and congestive heart failure, is provided by a therapy comprising use of a therapeutically-effective amount of spironolactone. Preferably, spironolactone is administered in the therapy at a low dose, that is, at a dose lower than has been conventionally used in clinical situations.

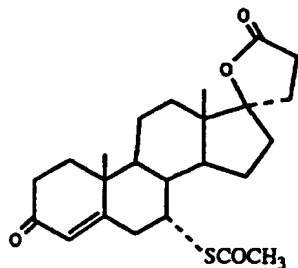
The therapy of the invention would be useful, for example, to prevent or retard, in a subject, the development of congestive heart failure which typically arises from essential hypertension or from heart conditions following myocardial infarct. Such subject would not typically be suffering from an edematous condition and thus would not gain benefit from treatment with conventional diuretic therapy as with loop diuretics which can alter electrolyte balance and cause hypokalemic or hypomagnesia conditions.

The phrase "aldosterone receptor antagonist" embraces an agent or compound, or a combination of two or more of such agents or compounds, which agent or compound binds to the aldosterone receptor as a competitive inhibitor of the action of aldosterone itself at an aldosterone receptor site, such as typically found in the renal tubules, so as to modulate the receptor-mediated activity of aldosterone. Typical of such aldosterone receptor antagonists are spiro lactone-type compounds. The term "spiro lactone-type" is intended to characterize a steroidal structure comprising a lactone moiety attached to a steroid nucleus, typically at the steroid "D" ring, through a spiro bond configuration.

The phrase "therapeutically-effective" is intended to qualify the amount of aldosterone receptor antagonist agent for use in the therapy which will achieve the goal of improvement in cardiac sufficiency by reducing or preventing, for example, the progression of congestive heart failure, while avoiding adverse side effects typically associated with such agent.

The phrase "low-dose amount", in characterizing a therapeutically-effective amount of the aldosterone receptor antagonist agent in the combination therapy, is intended to define a quantity of such agent, or a range of quantity of such agent, that is capable of improving cardiac sufficiency while reducing or avoiding one or more aldosterone-antagonist-induced side effects, such as hyperkalemia. A dosage of spironolactone which would accomplish the therapeutic goal of favorably enhancing cardiac sufficiency, while reducing or avoiding side effects, would be a dosage that substantially avoids inducing diuresis, that is, a substantially non-diuresis-effective dosage.

The compound spironolactone has the following structure and formal name:



"spironolactone": 17-hydroxy-7 $\alpha$ -mercapto-3-oxo-17 $\alpha$ -pregn-4-ene-21-carboxylic acid  $\gamma$ -lactone acetate

Methods to make spironolactone are described in U.S. Patent No. 3,013,012 to Cella et al which issued 12 December 1961. Spironolactone is sold by G.D. Searle &

Co., Skokie, Illinois, under the trademark "ALDACTONE", in tablet dosage form at doses of 25 mg, 50 mg and 100 mg per tablet.

5           A diuretic agent may be used in combination with spironolactone. Such diuretic agent may be selected from several known classes, such as thiazides and related sulfonamides, potassium-sparing diuretics, loop diuretics and organic mercurial diuretics.

10

          Examples of thiazides are bendroflumethiazide, benzthiazide, chlorothiazide, cyclothiazide, hydrochlorothiazide, hydroflumethiazide, methyclothiazide, polythiazide and trichlormethiazide.

15

          Examples of related sulfonamides are chlorthalidone, quinethazone and metolazone.

20           A diuretic agent may be used in combination with spironolactone. Such diuretic agent may be selected from several known classes, such as thiazides and related sulfonamides, potassium-sparing diuretics, loop diuretics and organic mercurial diuretics.

25

          Examples of thiazides are bendroflumethiazide, benzthiazide, chlorothiazide, cyclothiazide, hydrochlorothiazide, hydroflumethiazide, methyclothiazide, polythiazide and trichlormethiazide.

30

          Examples of sulfonamides related to thiazides are chlorthalidone, quinethazone and metolazone.

          Examples of potassium-sparing diuretics are triameterene and amiloride.

Examples of loop diuretics, i.e., diuretics acting in the ascending limb of the loop of Henle of the kidney, are furosemide and ethynacrylic acid.

- 5           Examples of organic mercurial diuretics are mercaptomerin sodium, merethoxylline procaine and mersaly with theophylline.

### Biological Evaluation

10

#### Human Clinical Trials

Spironolactone was evaluated in humans as described in the following clinical trials.

15

- Patients: Two-hundred fourteen (214) patients with symptomatic heart failure had an ejection fraction  $\leq 35\%$ , a history of New York Heart Association (NYHA) functional classification III-IV six months prior to enrollment, and  
20   current classification II-IV were randomized among five treatment groups. Patients were assigned to receive either spironolactone 12.5 mg (41 patients), 25 mg (45 patients), 50 mg (47 patients), 75 mg (41 patients), or placebo (40 patients) once a day for 12 weeks. Two patients that were  
25   randomized failed to take the study medication and were excluded from the analysis. All patients were taking a stable dose of ACE inhibitor, loop diuretic, and optional digitalis for 30 days prior to the first dose of study medication. Potassium supplement therapy that was stable  
30   for 14 days prior to the first dose of study medication was also allowed. Informed consent was obtained from all patients, and the protocol was approved by each ethical committee. At enrollment all patients had normal serum potassium values ( $< 5.5$  mmol/L) and creatinine values of  
35    $\leq 2.0$  mg/dL or  $\leq 180$  mmol/L. Patients were excluded from enrollment if they: (1) were diagnosed with either an acute life-threatening disease (included patients with

automatic implantable cardioverter/ defibrillator),  
valvular disease, unstable angina, insulin-dependent  
diabetes, cancer (without a reoccurrence within the last  
five years), or primary hepatic failure; (2) were on a  
5 waiting list for a heart transplant or experienced a  
myocardial infarction 30 days prior to the first dose of  
study medication; (3) had laboratory values for hematology  
or biochemistry considered abnormal and clinically  
significant prior to the first dose of study medication;  
10 (4) received a potassium spacing diuretic within 30 days  
prior to the first dose of study medication.; (5) were  
receiving, on a regular basis, either non-steroidal anti-  
inflammatory drugs or aspirin >325 mg/day, steroids,  
dopamine agonists or antagonists, insulin or heparin; (6)  
15 were on any investigational medication within 30 days of  
the first dose of medication.

Study Design: This was a multinational, double-blind,  
randomized, parallel group study.

20

Laboratory Measurements: The following information was  
obtained from each patient at baseline:

1. Concurrent medication within the past 30 days.
- 25 2. 12-lead ECG
3. Cardiac assessments that included blood pressure, pulse,  
sodium retention score (general assessment of a  
patient's edematous state was derived from the summation  
of scores obtained from Table I), NYHA classification,  
30 and
4. Signs and symptoms within the past 30 days.

Table I  
Sodium Retention Score

5	<u>Parameters</u>	<u>Grade</u>	<u>Assessment</u>
	Rales	0	Absent
		1	In lower 1/3 of lungs
		2	In lower 2/3 of lungs
		3	In all lung fields
10	Peripheral	0	Absent
	Pitting Edema	1	Trace
		2	Limited to ankles
		3	Not limited to ankles
15		4	Anasarca
	Weight Change	-1	Decreased
		0	Unchanged
		1	Increased
20	Hepatomegaly	0	Absent
		1	Present
	S3 Gallop	0	Absent
25		1	Present
	Increased Jugular	0	Absent
	Venous Pressure	1	Present
30	The following laboratory values were obtained at the pretreatment visit:		
	Hematology:	White blood cell count (WBC), hematocrit, hemoglobin, platelet count.	
35	Biochemistry:	Creatinine, potassium, AST, SGOT, urinary sodium/ potassium ratio, bicarbonate, calcium, chloride, creatinine, creatinine clearance, magnesium, glucose, urea, uric acid.	
40	Neurohormones:	Plasma renin activity, pro-atrial natriuretic factor, urinary aldosterone.	
45	Blood and urine samples were centrally analyzed at SciCor Laboratories. Laboratory values for urinary aldosterone and renin levels were done at the Ohio State University Laboratory in Columbus, Ohio. Pro-atrial natriuretic factor samples were evaluated at the University of Oslo		
50	Laboratory in Oslo, Norway. Patients were evaluated 9 days after beginning study medication. Documented changes in		

concurrent medications, signs and symptoms and drug compliance were recorded. These procedures were repeated at Week 4 and Week 8 visits. Patient information and procedures on the final visit (Week 12) was identical to the pre-treatment visit.

**Statistical Analysis:** Analysis of cardiac assessment changes in patient therapy and vital signs were performed for both the Intent-to-Treat (ITT) and evaluable patient groups. Analysis of demographic variables, adverse events and clinical laboratory values were performed in the ITT group. For each efficacy variable, results of each visit were examined separately. An appropriate trend test was used to test for overall dose-response. Pair-wise comparisons were made for each active dose to placebo. Significant levels for pair-wise comparisons were adjusted using the Hochberg-Bonferromi method to maintain the overall Type I error rate. All statistical methods were two-sided.

**Recruitment:** Two-hundred and fourteen patients were recruited from 22 study sites in eleven countries.

**Patient Characteristics:** Patient demographic, vital signs, and cardiac status at baseline are summarized in Table II.

TABLE II  
Patient Demographics

5	Demographic	Spironolactone 12.5 mg/d	Spironolactone 25 mg/d	Spironolactone 50 mg/d	Spironolactone 75 mg/d	Placebo	P- Value
10	Age (years)	63 ±12	61 ±9	62 ±13	62 ±13	61 ±12	N.S.
	Caucasian/other (%)	93/7	98/2	93/7	88/12	97/3	N.S.
	Male/female (%)	78/22	82/18	74/26	88/12	83/18	N.S.
15	<b>Vital Signs</b>						
	Weight (kg)	74	75	73	78	73	N.S.
	Blood pressure (mmHg)						
	Systolic	121	120	121	125	121	N.S.
	Diastolic	76	76	75	81	74	N.S.
	Pulse (bpm)	76	74	76	74	71	N.S.
20	<b>Cardiac Status</b>						
	NYHA (%)						
	II	63	60	43	49	38	
	III	34	38	55	49	60	
	IV	2	2	2	2	2	N.S.
	Sodium retention score	1.54	1.62	1.64	1.61	1.78	N.S.
25	Mean value						
	ACE-I (Mean dose)						
	Captopril (mg)	57.3	57.5	69.7	59.4	65.4	N.S.
	Enalapril (mg)	16.4	13.4	14.5	16.3	10.8	N.S.
30	Loop Diuretic (Mean dose)						
	Furosemide (mg)	58.8	82.8	76.9	84.9	63.2	N.S.
35	Digoxin (%)	78.0	77.8	76.6	80.5	77.5	N.S.
	Potassium supplement (%)	43.9	37.8	34.0	39.0	30.0	N.S.



Patients ranged in age from 26 to 83 years (mean = 60), 81% were male, 94% were Caucasian. At baseline 51% of the patients were NYHA Class II, 47% were Class III. With respect to sodium retention score, a statistically significant dose response was seen at Day 9 with higher doses showing more reduction in sodium retention score ( $p = 0.019$ ). However, this effect was not seen at later visits ( $p > 0.20$ ). There was an improvement in NYHA Class placebo group and in all the spironolactone groups. Although a trend toward improvement in the spironolactone group was observed, the difference was not statistically significant.

Changes in Patient Therapy: The treatment groups did not differ significantly with respect to changes in dose of ACE inhibitor, digitalis or potassium supplements at any visit ( $p \geq 0.11$ ). The treatment groups did differ significantly with respect to changes in loop diuretic therapy only at Week 8 ( $p = 0.004$ ) in that more patients on the higher doses of spironolactone had decreases in the loop diuretic dose compared to the placebo group. This pattern was not observed at Week 12.

Changes in Vital Signs: Changes from baseline in vital signs at Week 12 are summarized in Table III.

TABLE III  
Mean Change in Weight and Vital Signs from Baseline to Week 12

	Spironolactone 12.5 mg/d	Spironolactone 25 mg/d	Spironolactone 50 mg/d	Spironolactone 75 mg/d	Placebo	P- Value
10 Weight	0.59 (3.00)	-0.16 (3.02)	0.62 (2.05)	-0.81 (2.70)	0.11 (2.46)	0.109
Supine systolic BP	1.84 (11.82)	-4.46 (13.97)	-7.04 (15.83)	-5.68 (15.62)	0.22 (13.45)	0.036
Supine diastolic BP	-0.19 ( 9.13)	-2.74 ( 9.57)	-5.11 (11.11)	-5.91 (9.05)	1.78 (7.84)	0.014
15 Supine pulse (BPM)	-3.70 ( 9.56)	-1.40 (10.00)	-3.21 (11.27)	-1.07 (13.79)	1.42 (9.69)	0.422

At all visits the 25 mg, 50 mg, and 75 mg groups had decreases in mean systolic and diastolic blood pressure, while the placebo group had increases in mean systolic and diastolic blood pressure (both standing and supine). Dose response with respect to standing and supine diastolic blood pressure was statistically significant for all visits ( $p \leq 0.002$ ). Dose response with respect to standing and supine blood pressure was statistically significant at Week 4, Week 8, and Week 12 ( $p \leq 0.033$ ), but not at Day 9 ( $p \geq 0.12$ ). No significant between-treatment differences in change from baseline in pulse were observed at any visit ( $p$ -values  $\geq 0.136$ ). A statistically significant dose response with greater decreases in pulse in the supine position at higher doses was observed at Week 4 ( $p$ -value = 0.045). Spironolactone doses of 25 and 50 mg were also significantly different from placebo ( $p$ -values  $\leq 0.043$ ) (See Fig. 1). At Day 9 and Week 4 visits, there was a statistically significant dose response with respect to changes from baseline in body weight in that patients in the 75 mg dose group experienced more weight loss than other patients. This dose response was not observed at later visits ( $p \geq 0.062$ ).

25

**Clinical Laboratory Values:** Table IV contains details of the different clinical laboratory values that showed statistically significant treatment differences with respect to mean changes at Week 12 visit compared with their respective baseline value.

30

TABLE IV  
Week 12 Mean Change

		Spiro 12.5 mg/d	Spiro 25 mg/d	Spiro 50 mg/d	Spiro 75 mg/d	Placebo	p- value
5							
	Urinary aldosterone (nmol/D)	4.21	4.27	8.11	11.13	0.76	0.002
10	N-Terminal ANF (pmol/L)	-287.30	-294.60	-351.30	-370.60	54.50	0.022
	PRA (NgAngl/L/s)	9.90	9.33	13.18	10.23	0.50	0.002
15							
	Hematocrit (%)	0.00	-0.02	-0.02	-0.03	0.00	0.002
	Hemoglobin (mmol/L/Fe)	0.12	-0.20	-0.31	-0.46	0.00	0.005
	Potassium (mmol/L)	0.18	0.37	0.51	0.58	-0.10	0.001
20							
	Creatinine (umol/L)	6.83	9.30	14.06	21.90	-1.96	0.001
	Sodium (mmol/L)	-1.61	-1.85	-2.52	-3.37	-0.03	0.001

Urinary Aldosterone (See Fig. 2): Urinary aldosterone was determined only for baseline and the 12 week visit.

- 5 Urinary aldosterone excretion showed mean increases from baseline in all treatment groups ( $P \leq 0.012$ ). Greater increases were seen at higher doses of spironolactone ( $p = 0.002$ ). All pair-wise comparisons between active treatment and placebo were statistically significant ( $p \leq 0.009$ ).

10

Plasma Renin Activity (PRA) (See Fig. 2): A

- statistically significant dose-response with respect to change from baseline in PRA was seen at Day 9, Week 4 and  
15 Week 12 ( $P \leq 0.001$ ) with higher doses of spironolactone associated with greater increases in PRA. PRA was not measured at Week 8.

N-Terminal Atrial Natriuretic Factor (ANF) (See

- 20 Fig. 3): All active treatments showed decreases from baseline at all treatment visits. Dose-response was statistically significant at Day 9 ( $p = 0.048$ ), Week 4 ( $p = 0.005$ ), and Week 12 ( $p = 0.008$ ). ANF was not measured at Week 8. In comparisons the 50 mg dose group differed  
25 significantly from placebo at Week 4 ( $p = 0.009$ ) and Week 12 ( $p = 0.006$ ), while the 75 mg dose group differed significantly from placebo at Week 12 only ( $p = 0.007$ ).

Hematocrit and Hemoglobin: At Day 9 visit a

- 30 statistically significant mean value difference between placebo and the different active treatments was observed with lower values for the placebo group than the active treatments ( $p < 0.001$ ). At Week 12 a reverse statistically significant difference was observed with lower levels for  
35 the active treatment groups for hematocrit ( $p = 0.002$ ) and hemoglobin (0.005).

**Serum Potassium:** A statistically significant dose-response with respect to change from baseline in serum potassium was seen at all treatment period visits ( $p < 0.001$ ). Higher doses of spironolactone were associated with larger increases in potassium. All doses of active treatment had significantly higher serum potassium levels relative to baseline than placebo ( $p \leq 0.034$ ).

#### **Incidence of Hyperkalemia**

10	Treatment: Placebo	Spironolactone 12.5 mg/d	Spironolactone 25 mg/d	Spironolactone 50 mg/d	Spironolactone 75 mg/d
	Patients (%)	2(5%)	6(13%)	9(20%)	10(24%)

15 **Predictors of Hyperkalemia:** Seven possible predictors of hyperkalemia (potassium  $\geq 5.5$  mmol/L) were included in a step-wise Cox regression analysis: randomized treatment (treated as a categorical variable), age, baseline NYHA class, baseline serum potassium, baseline PRA, baseline creatinine, baseline urinary aldosterone, and type and dose of ACE-I. Besides the dose of spironolactone, the following predictors of hyperkalemia were statistically significant in the step-wise regression analysis: type of  
20 ACE-I (captopril versus other), baseline serum creatinine, and baseline serum potassium. Results are summarized as follows:

30	<b><u>Factor</u></b>	<b><u>p-value</u></b>	<b><u>Risk Ratio</u></b>
	Captopril vs other ACE-I	0.013	0.318
	Serum Creatinine > normal	0.038	2.72
	Baseline Potassium > median	0.040	2.32

35 In this analysis, the risk ratio can be thought of as the probability that the patient with the risk factor will develop hyperkalemia, relative to the probability that a patient without the risk factor will develop it. (For example, patients on captopril are about one-third as likely  
40 to develop hyperkalemia as a patient on another ACE-I.)

Risk ratios relative to placebo for the various doses of spironolactone are:

	<u>Dose</u>	<u>p-value</u>	<u>Risk Ratio</u>
5	Spironolactone 12.5 mg	0.98	1.02
	Spironolactone 25 mg	0.19	2.91
	Spironolactone 50 mg	0.034	5.32
	Spironolactone 75 mg	0.016	6.66

After adjusting for the above factors, other predictors  
10 included in the step-wise regression analysis were not  
significant ( $p$ -values  $\geq 0.07$ ). However, the following  
additional factor was significantly related to the  
development of hyperkalemia when considered apart from  
other predictors except the dose of spironolactone.

15	<u>Factor</u>	<u>p-value</u>	<u>Risk Ratio</u>
	High ACE-I Dose	0.050	2.93

Serum Magnesium: Change from baseline in serum  
20 magnesium showed a statistically significant dose-response  
at Day 9 and Week 4 ( $p \leq 0.048$ ), with more patients in the  
placebo group showing decreases in serum magnesium.  
However, this effect was not seen at later visits ( $p$   
 $\geq 0.083$ ).

25 Adverse Effects: Table V summarizes the twelve most  
common adverse events by different treatment groups. Only  
one symptom, hyperkalemia, showed a clear dose-response in  
term of incidence ( $p = 0.001$ ).

Table V  
Incidence of Adverse Events  
Spironolactone Dose-Ranging Study  
Intent-to-Treat Cohort  
(Top Twelve Events)

	Adverse Events	Treatment Group (Percentage of Patients)					Total
		Spironolactone 12.5 mg	Spironolactone 25 mg	Spironolactone 50 mg	Spironolactone 75 mg	Placebo	
15	Dyspnea	22.0	15.6	26.1	24.4	30.0	23.5
	Angina Pectoris	19.5	20.0	8.7	14.6	17.5	16.0
	Dizziness	12.2	13.3	13.0	17.1	15.0	14.1
	Fatigue	12.2	13.3	15.2	14.6	15.0	14.1
	Nausea	2.4	17.8	6.5	19.5	12.5	11.7
20	Diarrhea	4.9	22.2	8.7	14.6	5.0	11.3
	Abdominal Pain	7.3	8.9	13.0	7.3	17.5	10.8
	Headache	9.8	2.2	15.2	7.3	20.0	10.8
	Hyperkalemia	2.4	8.9	15.2	19.5	2.5	9.9
25	URT Infection	4.9	11.1	8.7	2.4	12.5	8.0
	Arthralgia	4.9	4.4	8.7	4.9	7.5	6.1
	Coughing	4.9	2.2	4.3	2.4	12.5	5.2



A breakdown of the hospitalizations is as follows:

	<u>Treatment</u>	<u>Placebo</u>	<u>12.5 mg</u>	<u>25 mg</u>	<u>50 mg</u>	<u>75 mg</u>	<u>P</u>
5	Patients (%)	5(12.5%)	3(7.3%)	3(6.6%)	13(27.6%)	6(14.6%)	N.S.

No deaths were reported during the drug treatment period. Three patients died within 30 days after the study was completed. These three patients were previously at the 50  
10 mg dose.

Administration of spironolactone may be accomplished by oral route, or by intravenous, intramuscular or subcutaneous injections. If a diuretic is used in co-  
15 therapy, with spironolactone, then administration may take place sequentially in separate formulations, or may be accomplished by simultaneous administration in a single formulation or separate formulations. The formulation may be in the form of a bolus, or in the form of aqueous or  
20 non-aqueous isotonic sterile injection solutions or suspensions. These solution and suspensions may be prepared from sterile powders or granules having one or more pharmaceutically-acceptable carriers or diluents, or a binder such as gelatin or hydroxypropyl-methyl cellulose,  
25 together with one or more of a lubricant, preservative, surface-active or dispersing agent.

For oral administration, the pharmaceutical composition may be in the form of, for example, a tablet,  
30 capsule, suspension or liquid. The pharmaceutical composition is preferably made in the form of a dosage unit containing a particular amount of the active ingredient. Examples of such dosage units are tablets or capsules. A suitable daily dose for a mammal may vary widely depending  
35 on the condition of the patient and other factors. Spironolactone may be present in an amount of from about 1 to 400 mg, preferably from about 2 to 150 mg, depending upon the specific ALDO antagonist compound selected and the specific disease state being targetted for the combination

therapy. If a diuretic agent is used in co-therapy, then such agent may be present in an amount from about 1 mg to about 400 mg per dose, and more preferably from about 1 mg to about 150 mg per dose, depending on the diuretic  
5 selected.

For disease states which require prevention, reduction or treatment of a cardiovascular disease state without incidence of hyperkalemia, for example, spironolactone will  
10 be present in the therapy in an amount in a range from about 1 mg to about 25 mg per dose. A preferred range for spironolactone would be from about 5 mg to 15 mg per dose. More preferably would be a range from about 10 mg to 15 mg per dose per day.

15

Examples of various ALDO antagonist daily doses of the invention are as follow:

	<u>Spironolactone (mg)</u>
20	5
	7.5
	10
	12.5
	15
25	17.5
	20
	22.5

The active ingredients may also be administered  
30 by injection as a composition wherein, for example, saline, dextrose or water may be used as a suitable carrier.

The dosage regimen for treating a disease condition with the combination therapy of this invention is  
35 selected in accordance with a variety of factors, including the type, age, weight, sex and medical condition of the patient, the severity of the disease, the route of administration, and the particular compound employed, and

thus may vary widely.

For therapeutic purposes, the active components of this combination therapy invention are ordinarily combined with one or more adjuvants appropriate to the indicated route of administration. If administered per os, the components may be admixed with lactose, sucrose, starch powder, cellulose esters of alkanolic acids, cellulose alkyl esters, talc, stearic acid, magnesium stearate, magnesium oxide, sodium and calcium salts of phosphoric and sulfuric acids, gelatin, acacia gum, sodium alginate, polyvinylpyrrolidone, and/or polyvinyl alcohol, and then tableted or encapsulated for convenient administration. Such capsules or tablets may contain a controlled-release formulation as may be provided in a dispersion of active compound in hydroxypropylmethyl cellulose. Formulations for parenteral administration may be in the form of aqueous or non-aqueous isotonic sterile injection solutions or suspensions. These solutions and suspensions may be prepared from sterile powders or granules having one or more of the carriers or diluents mentioned for use in the formulations for oral administration. The components may be dissolved in water, polyethylene glycol, propylene glycol, ethanol, corn oil, cottonseed oil, peanut oil, sesame oil, benzyl alcohol, sodium chloride, and/or various buffers. Other adjuvants and modes of administration are well and widely known in the pharmaceutical art.

Pharmaceutical compositions for use in the treatment methods of the invention may be administered in oral form or by intravenous administration. Oral administration of the therapy is preferred. Dosing for oral administration may be with a regimen calling for single daily dose, or for a single dose every other day, or for multiple, spaced doses throughout the day. Where a combination therapy is desired, for example, an aldosterone antagonist and a diuretic, the active agents which make up the combination therapy may be administered simultaneously, either in a

combined dosage form or in separate dosage forms intended for substantially simultaneous oral administration. The active agents which make up the combination therapy may also be administered sequentially, with either active component being administered by a regimen calling for two-step ingestion. Thus, a regimen may call for sequential administration of the active agents with spaced-apart ingestion of the separate, active agents. The time period between the multiple ingestion steps may range from a few minutes to several hours, depending upon the properties of each active agent such as potency, solubility, bioavailability, plasma half-life and kinetic profile of the agent, as well as depending upon the age and condition of the patient. The active agents of the combined therapy whether administered simultaneously, substantially simultaneously, or sequentially, may involve a regimen calling for administration of one active agent by oral route and the other active agent by intravenous route. Whether the active agents of the combined therapy are administered by oral or intravenous route, separately or together, each such active agent will be contained in a suitable pharmaceutical formulation of pharmaceutically-acceptable excipients, diluents or other formulations components. Examples of suitable pharmaceutically-acceptable formulations containing the active components for oral administration are given below. Even though such formulations list both active agents together in the same recipe, it is appropriate for such recipe to be utilized for a formulation containing one of the active components.

30

#### Example 1

An oral dosage may be prepared by screening and then mixing together the following list of ingredients in the amounts indicated. The dosage may then be placed in a hard gelatin capsule.

35

	<u>Ingredients</u>	<u>Amounts</u>
	spironolactone	12.5 mg
	magnesium stearate	10 mg
5	lactose	100 mg

Example 2

10 An oral dosage may be prepared by mixing together granulating with a 10% gelatin solution. The wet granules are screened, dried, mixed with starch, talc and stearic acid, screened and compressed into a tablet.

	<u>Ingredients</u>	<u>Amounts</u>
15	spironolactone	12.5 mg
	calcium sulfate dihydrate	100 mg
	sucrose	15 mg
	starch	8 mg
	talc	4 mg
20	stearic acid	2 mg

Example 3

25 An oral dosage may be prepared by screening and then mixing together the following list of ingredients in the amounts indicated. The dosage may then be placed in a hard gelatin capsule.

	<u>Ingredients</u>	<u>Amounts</u>
30	spironolactone	12.5 mg
	furosemide	73.9 mg
	magnesium stearate	10 mg
	lactose	100 mg

Example 4

35 An oral dosage may be prepared by mixing together granulating with a 10% gelatin solution. The wet granules are screened, dried, mixed with starch, talc and stearic  
40 acid, screened and compressed into a tablet.

	<u>Ingredients</u>	<u>Amounts</u>
	spironolactone	12.5 mg
	furosemide	73.9 mg
5	calcium sulfate dihydrate	100 mg
	sucrose	15 mg
	starch	8 mg
	talc	4 mg
10	stearic acid	2 mg

Although this invention has been described with respect to specific embodiments, the details of these embodiments are not to be construed as limitations.

What Is Claimed Is:

1. A pharmaceutical dosage comprising one or more pharmaceutically-acceptable excipients and spironolactone in an amount which is therapeutically effective to antagonize aldosterone but which amount is not sufficient to induce substantial diuresis.
2. The dosage of Claim 1 wherein said subject is afflicted with or susceptible to congestive heart failure and said subject further requires avoidance of the incidence of hyperkalemia.
3. The dosage of Claim 1 wherein spironolactone is present in a dose range from about 1 mg to about 23 mg.
4. The dosage of Claim 3 wherein spironolactone is present in a dose range of about 5 mg to about 20 mg.
5. The dosage of Claim 4 wherein spironolactone is present in a dose of about 12.5 mg.
6. A method to treat a circulatory disorder in a subject in need thereof, said method comprising administering to said subject an amount of spironolactone sufficient to prevent or retard the development of congestive heart failure but less than an amount to induce incidence of hyperkalemia.
7. The method of Claim 6 comprising administering spironolactone in an amount that substantially avoids inducing diuresis.
8. The method of Claim 7 comprising administering spironolactone in an amount less than 25 mg per day.

9. The method of Claim 8 comprising administering spironolactone in an amount from about 5 mg to about 20 mg per day.

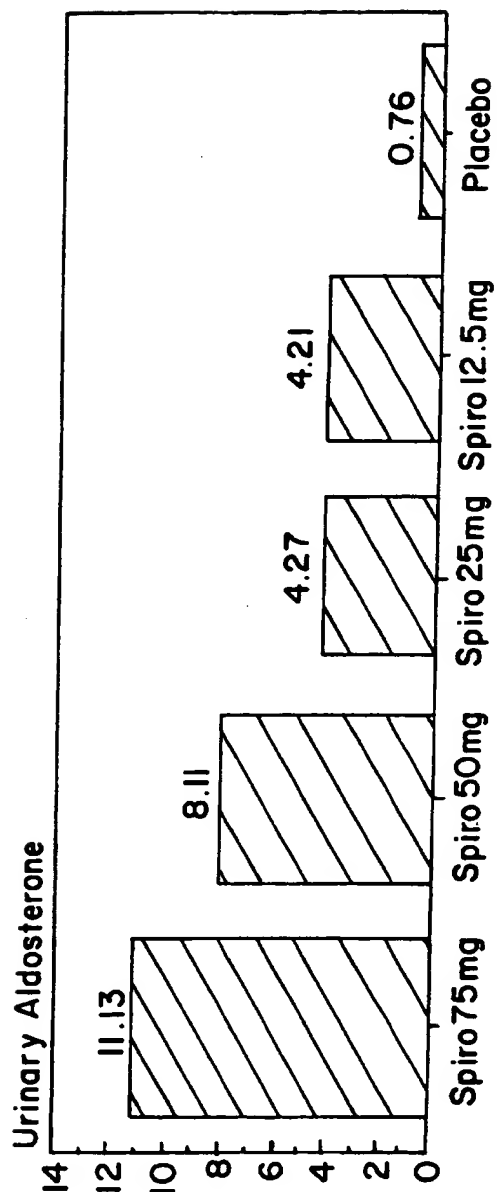
5 10. The method of Claim 8 comprising administering spironolactone in an amount from about 5 mg to about 15 mg per day.

10 11. The method of Claim 8 comprising administering spironolactone in an amount of about 12.5 mg per day.

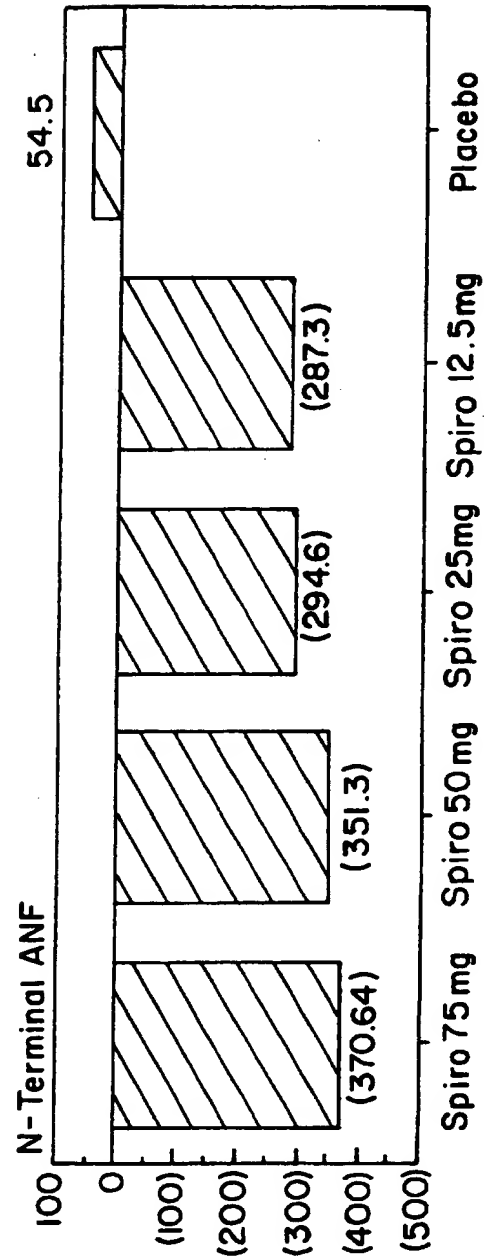
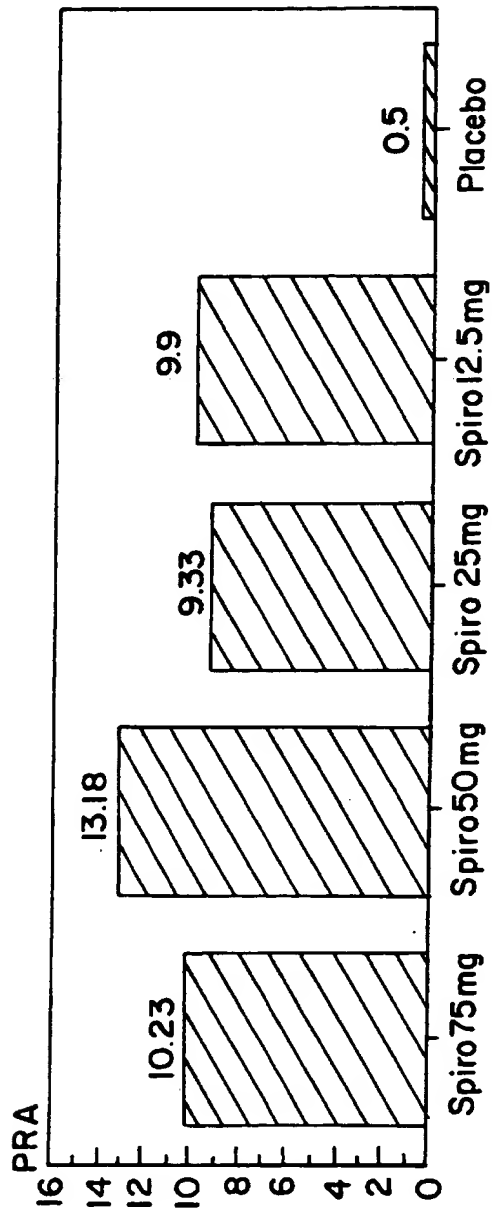


1 / 3

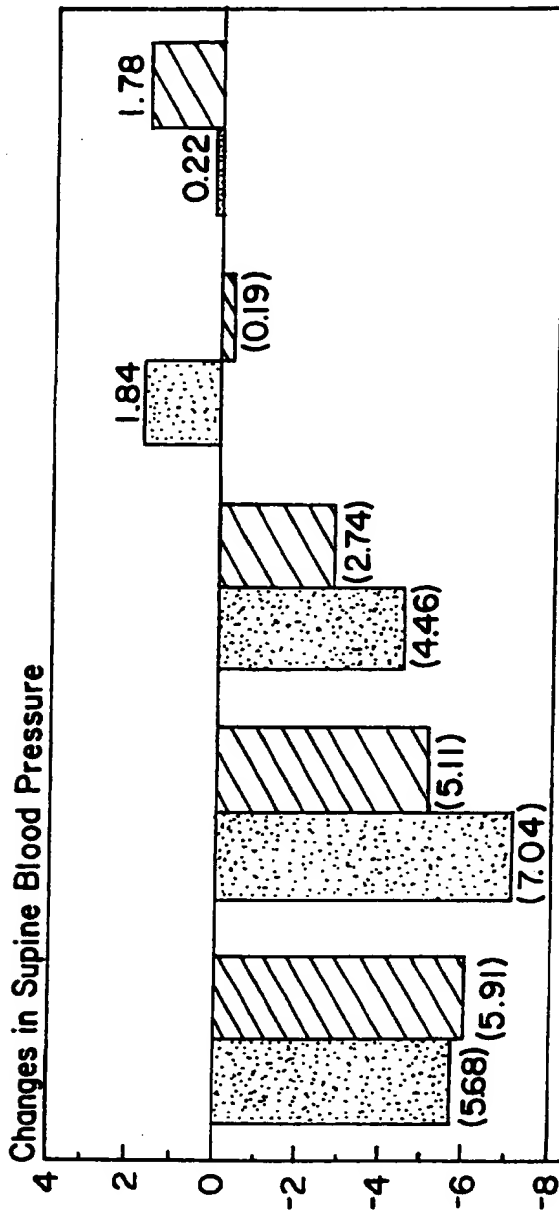
FIG. 1



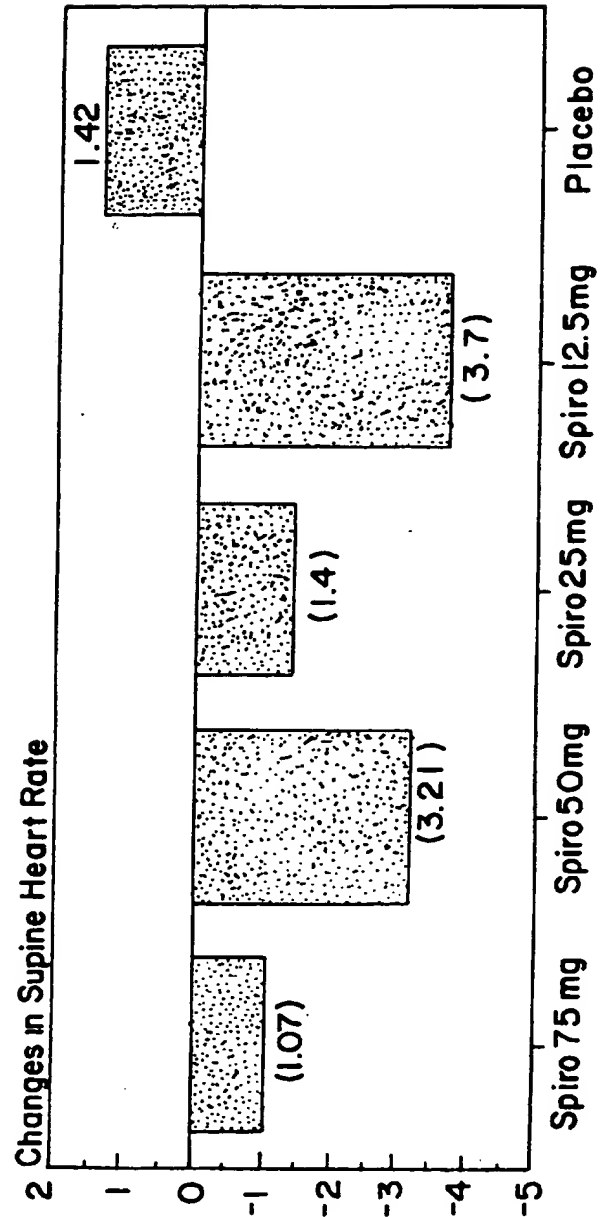
2 / 3



3 / 3



■ Supine Systolic  
 ▨ Supine Diastolic



# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 96/01769

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K31/585

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BE,A,873 092 (E.R.SQUIBB & SONS, INC.) 27 June 1979 see page 4, line 21 - line 35 ---	1-5
X	DE,A,20 61 698 (E. ENGELKE GEB. RÖSSNER) 6 July 1972 see the whole document ---	1-5
X	DE,A,26 03 890 (MERCK & CO., INC.) 5 August 1976 see the whole document ---	1-5
X	EP,A,0 410 348 (G.D.SEARLE & CO.) 30 January 1991 see the whole document ---	1-5
	--- -/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "A" document member of the same patent family

Date of the actual completion of the international search

11 June 1996

Date of mailing of the international search report

21.06.96

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Theuns, H

# INTERNATIONAL SEARCH REPORT

Inter national Application No

PCT/US 96/01769

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO,A,95 09625 (HANDFORTH INVESTMENTS LTD.) 13 April 1995 see page 4, line 1 - line 6 ---	1-5
X,P	WO,A,95 17169 (APPLIED ANALYTICAL INDUSTRIES, INC.) 29 June 1995 see page 3 ---	1-5
X	GB,A,2 113 544 (MAYRON INTERNATIONAL INC.) 10 August 1983 see the whole document ---	1-5
X,P	WO,A,95 15166 (CURATORS OF THE UNIVERSITY OF MISSOURI) 8 June 1995 see the whole document ---	1-11
X	"farmacotherapeutisch kompas 1984" 1984 , CENTRALE MEDISCH PHARMACEUTISCHE COMMISSIE VAN DE ZIEKENFONDSRAAD XP002005312 see page 402 - page 403 -----	1-5

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 96/01769

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claims 6-12 are directed to a method of treatment of the  
human/animal body, the search has been carried out and based on the alleged  
effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such  
an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all  
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment  
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report  
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is  
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International Application No  
PC1/US 96/01769

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
BE-A-873092	27-06-79	AR-A- 230378	30-04-84
		AU-B- 526239	23-12-82
		AU-B- 4204178	05-07-79
		CA-A- 1120400	23-03-82
		CH-A- 642542	30-04-84
		DE-A- 2854316	05-07-79
		FR-A,B 2413089	27-07-79
		GB-A,B 2010675	04-07-79
		JP-B- 1007045	07-02-89
		JP-C- 1523601	12-10-89
		JP-A- 54110333	29-08-79
		LU-A- 80709	13-04-79
		NL-A- 7812164	29-06-79
		SE-B- 445173	09-06-86
		SE-A- 7813277	28-06-79
		US-A- 4217347	12-08-80
-----			
DE-A-2061698	06-07-72	NONE	
-----			
DE-A-2603890	05-08-76	AU-B- 507494	14-02-80
		AU-B- 1061176	04-08-77
		BE-A- 838170	02-08-76
		CA-A- 1075155	08-04-80
		FR-A,B 2299025	27-08-76
		GB-A- 1501592	15-02-78
		NL-A- 7600500	05-08-76
-----			
EP-A-0410348	30-01-91	AU-B- 5974390	31-01-91
		CA-A- 2021872	27-01-91
		JP-A- 3095120	19-04-91
-----			
WO-A-9509625	13-04-95	AU-B- 7854094	01-05-95
-----			
WO-A-9517169	29-06-95	ZA-A- 9309566	11-08-94
		AU-B- 1439895	10-07-95
-----			
GB-A-2113544	10-08-83	NONE	
-----			
WO-A-9515166	08-06-95	AU-B- 1210695	19-06-95
-----			